REMARKS

Applicants' representatives wish to thank the Examiner for the interview of March 7, 2003. After the Examiner has reviewed the present response, the Examiner is invited to call the Applicants' representative if a telephone conference would expedite prosecution.

Status of the Claims

Claims 1-20 and 58-92 are pending in the present application. Claims 78 and 89 have been cancelled without prejudice or disclaimer. Claim 12 has been amended to correctly designate the nucleotides of SEQ ID NO:1 that show the hepatitis B virus EnhI enhancer. Support for the amendment may be found on page 6, line 11 and page 28, line 16 of the specification. Claim 18 has been amended to recite specific conditions for high stringency hybridization. Support for this amendment may be found on line 30 of page 21 through line 4 of page 22 of the specification. Claims 1, 9, 18, 58, 68, 73, 80, and 82 have been amended to recite that the recited vectors and nucleic acid molecules comprise a heterologous nucleotide sequence encoding B-domain deleted factor VIII operably linked with at least on enhancer and an AAV ITR, wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAF ITR. Support for these amendments may be found in original claim 7 and on lines 11-13 of page 18 of the specification. Claim 9 has been amended to recite that the claimed rAAV vector comprises a liver-preferred enhancer. Support for the amendment may be found on lines 25-26 of page 27 of the specification. Claim 58 has been amended to recite that the claimed nucleic acid molecule comprises a hepatitis virus enhancer. Support for the amendment may be found on lines 26-28 of page 5 of the specification. New claims 91 and 92 have been added. Support for these claims may be found in original claims 1 and 7, and in the specification on lines 17-18 of page 3, lines 1-3 of page 9, lines 3-9 of page 15, and lines 14-18 of page 18. Claims 59-65, and 74-77, and 79 have been amended to correct formal matters. No new matter has been added by way of amendment. Reexamination and reconsideration of the claims are respectfully requested.

The Rejection Under 35 U.S.C. § 112, First Paragraph, Should be Withdrawn

Claims 18, 19, 35, and 68-90 have been rejected under 35 U.S.C. § 112, first paragraph, on the grounds that these claims are drawn to subject matter that was not described in such a way as to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention at the time the application was filed. The rejection is respectfully traversed for the reasons described below.

It is the Applicants' understanding that the Examiner indicated during the telephone interview on March 7, 2003, that the rejection under 35 U.S.C. § 112, first paragraph, would with withdrawn in view of the number of species of B-domain deleted factor VIII disclosed in the specification. The following arguments are presented to complete the record and to fully respond to the rejection

In the Amendment mailed June 12, 2002, the Applicants presented arguments demonstrating that the subject matter of claims 18, 19, 35, 68-79, and 82-90 met the requirements for written description under 35 U.S.C. § 112, first paragraph. These arguments are herein incorporated by reference. In the Office Action mailed September 11, 2002 the Examiner states the Applicants' arguments were not found persuasive, and that the written description of the claimed invention is insufficient because "the claims embrace [a] large amount of other sequences non-described, and there is no known or disclosed correlation between the function of B-domain deleted factor VIII and the structure of non-described nucleic acids that are 75-95% identical to 419-4835 of the SEQ ID NO:1." September 11, 2002 Office Action, page 4.

As an initial matter, Applicants note that claims 80 and 81 do not encompass variants of the disclosed sequences and therefore the arguments presented in the office action are not applicable to these claims. Furthermore, the specification cites and incorporates references which disclose species of B-domain deleted factor VIII falling within the scope of the claims and provide guidance regarding regions of B-domain deleted factor VIII that are required for its biological activity. These references include Pittman *et al.* (1993) *Blood* 11:2925, Gnatenko *et al.*, (1999) *Br. J. Haemotology* 104:27, Ill *et al.* (1997) *Blood Coagulation and Fibrinolysis* 8:S23, and U.S. Patent 5,910,481. *See,* lines 23-30 of page 20 of the specification. Thus,

contrary to the statement made in the office action, domains required for the biological activity of B-domain deleted factor VIII are described in the specification.

In addition, the standard for written description set forth in the office action is inconsistent with the "Guidelines for Examination of Patent Applications Under the 35 U.S.C. 112, ¶ 1, 'Written Description' Requirement," 66 Fed. Reg. 1099 (2001), and the supporting case law. The "Guidelines" state that the written description requirement may be met by "show[ing] that an invention is complete by disclosure of sufficiently detailed, relevant, identifying characteristics . . . i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, ***or*** some combination of such characteristics." 66 Fed. Reg. at 1106, *emphasis added.* Thus, the guidelines require that a correlation between structure and function be disclosed when a claimed invention is described solely by its functional characteristics, but do not require such a correlation when the claimed invention is described by complete or partial structure.

In the present case, the Applicants have not relied solely on functional characteristics to describe the claimed genera of sequences. Rather, claims 18, 19, 35, 68-79, and 82-90 recite the identifying structural characteristics that define each genus of nucleotide sequences. Specifically, these claims recite nucleotide sequences having at least 75%, 80%, 85%, 90%, or 95% sequence identity with nucleotides 419-4835 of SEQ ID NO:1, and nucleotide sequences that hybridize to nucleotides 419-4835 of SEQ ID NO:1 under defined conditions. Accordingly, claims 18, 19, 35, 68-79, and 82-90 meet the standard set forth in the "Guidelines."

Claims 18, 19, 35, 68-79, and 82-90 also meet the standard for written description set forth in *Regents of the University of California v. Eli Lilly & Co*, 119 F.3d 1559 (Fed. Cir. 1997), where the court held that "[a] written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula, or chemical name' of the claimed subject matter sufficient to distinguish it from other materials." 119 F.3d at 1568, citing Fiers v. Revel 984 F.2d 1164 (Fed. Cir. 1993). The structural limitations recited in claims 18, 19, 35, and 68-79, and 82-90 are sufficient to

distinguish the claimed nucleotide sequences from other materials and thus sufficiently define the claimed genus.

The Federal Circuit in *Lilly* further held that "[a] description of a genus of cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus." 119 F.3d at 1569. The written description provided for the genera of sequences recited in claims 18, 19, 35, and 68-79, and 82-90 meets this requirement because these claims recite the identifying structural characteristics that define each genus of polynucleotides. Specifically, these claims encompass only those nucleotide sequences having at least 75%, 80%, 85%, 90%, or 95% sequence identity with nucleotides 419-4835 of SEQ ID NO:1, and nucleotide sequences that hybridize to nucleotides 419-4835 of SEQ ID NO:1 under defined conditions..

Applicants note that an absolute requirement for a correlation between the functional characteristics and structural characteristics of a genus of sequences set forth in the Office Action is at odds with the "Revised Interim Written Description Guidelines Training Materials" available at www.uspto.gov/web/menu/written.pdf. Example 14 of the "Training Materials" provides a written description assessment for a claim to a protein having at least 95% sequence identity to the sequence of SEQ ID NO:3, wherein the sequence catalyzes the reaction A → B. The analysis of Example 14 in the Training Materials does not conclude that the genus of sequences is insufficiently described because the specification does not demonstrate the structural motifs underlying the function of the claimed polypeptide. Rather, the conclusion in the Training Materials is that the generic claim of Example 14 is sufficiently described under §112, first paragraph, because 1) "the single sequence disclosed in SEQ ID NO:3 is representative of the genus" and 2) the claim recites a limitation requiring the compound to catalyze the reaction from A → B, and therefore one of skill in art would recognize that the Applicants were in possession of the necessary common attributes possessed by the members of the genus.

Similarly, claims 19, 21, 24, 26, 27, 29, 32, and 33 provide the relevant, identifying characteristics that describe the claimed genera of sequences, and one of skill in the art would recognize that the inventors were in possession of the claimed invention. Therefore, the requirement for a written description of the claimed invention under 35 U.S.C. §112, first paragraph, is met.

The Examiner cites several references in support of the rejection for inadequate written description. The *Encyclopedia Britannica online* is cited for the teaching that "[s]ince each position in a peptide is uniquely defined, the number of possible peptides is very large, even in a relatively short peptide . . . the preparation of a specific peptide sequence and the determination of the sequence of the amino acids in a peptide protein chain requires specifically adapted chemical methods." Applicants note that the present invention is not directed to methods of preparing specific peptide sequences, or to methods of determining protein sequence. Furthermore, the instant claims do not encompass nucleotide sequences encoding every possible polypeptide having the same length as the polypeptide encoded by nucleotides 419 to 4835 of SEQ ID NO:1. Rather, claims 18, 19, 35, and 68-79, and 82-90 encompass only those nucleotide sequences having at least 75%, 80%, 85%, 90%, or 95% sequence identity with nucleotides 419-4835 of SEQ ID NO:1, and nucleotide sequences that hybridize to nucleotides 419-4835 of SEQ ID NO:1 under defined conditions. Accordingly, the reference is not relevant to the sufficiency of the written description provided for the subject matter of these claims.

The Examiner cites Bowie *et al.* (1990) *Science* 247:1306-10 for the teaching that prediction of a protein's 3-dimensional structure based on its sequence is extremely complex, and that mutations in residues of a protein that are required for structure formation can have dramatic effects on protein activity. As an initial matter, Applicants note that the present application is not directed to proteins having any particular 3-dimensional structure. Furthermore, Bowie *et al.* report that "[s]tudies . . . have revealed that proteins are surprisingly tolerant of amino acid substitutions . . . [f]or example, in studying the effects of approximately 1500 single amino acid substitutions at 142 positions in *lac* repressor, Miller and co-workers found that about one-half of all substitutions were phenotypically silent." Bowie *et al.* page 1306, column 2. Thus, according

to the teachings of Bowie *et al.*, many of the variants of B-domain deleted factor VIII that meet structural limitations recited in the claims will also be biologically active.

The Examiner also cites Everett *et al.* (1997) *Nature Genetics* 17:411-22 and Scott *et al.* (1999) *Nature Genetics* 21:440-43, in support of the rejection for insufficient written description. Everett *et al.* teach the positional cloning of the genetic defect that causes Pendred syndrome. The encoded gene, pendrin, shares sequence similarity with sulfate transporters and was predicted by Everett *et al.* to function as a sulfate reporter. Scott *et al.* demonstrate that pendrin does function as an anion transporter, but transports chloride and iodide rather than sulfate. Taken together, the references demonstrate that sequence similarity was useful in predicting the general functional class to which the pendrin protein belonged (i.e. anion transporters), but that additional research was required to determine precisely which anions were transported by the pendrin protein. The teachings of these references are not relevant to the written description of the present invention, because the rejected claims are not directed to sequences of a novel protein whose function has been determined *de novo* based on sequence similarity. Rather, the present invention is directed to variants of B-domain deleted factor VIII, a protein having a known biochemical and biological function. Furthermore, domains required for the biological activity of B-domain deleted factor VIII are disclosed in the specification as described above. The specification also provides guidance regarding conservative substitutions of amino acids. *See*, lines 3-16 of page 23. In addition, the B-domain deleted factor VIII variants encompassed by the claims are defined by structural limitations. Accordingly, the cited references do not support the rejection of claims 18, 19, 35, and 68-79, and 82-90 for insufficient written description.

The Examiner also cites Bork (2000) *Genome Res.* 10:398-400 in support of the rejection for insufficient written description. The Bork reference, like the Everett *et al.* and Scott *et al.* references discussed above, is directed to the use of sequence analysis to determine function. Specifically, Bork *et al.* discusses the error rates for methods of predicting the location of promoters, regulatory RNA elements, alternative splicing sites, three dimensional protein structure features, protein function, and protein cellular localization based on sequence analysis. The Examiner specifically cites the first column of page 400, which teaches that predictions of

protein-protein interactions in yeast based on sequence have a high error rate. However, the teachings of the Bork reference are not relevant to the written description of the B-domain deleted variants of claims 18, 19, 35, and 68-79, and 82-90, because these claims are not directed to promoters, regulatory RNA elements, alternative splicing sites, or to proteins having three dimensional structural features, cellular localizations, or functions based solely on sequence analysis. Rather, the claims are directed to variants of a protein having known function, where domains required for protein function and guidance regarding conservative substitution of amino acids are given in the specification. Accordingly, the Bork reference does not support the rejection of claims 18, 19, 35, and 68-79, and 82-90 for insufficient written description.

The rejection of claims 18, 19, and 35 under 35 U.S.C. §112, first paragraph, has been maintained on the grounds that the specification, while being enabling for making and using an rAAV vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII, does not reasonably provide enablement for rAAV vectors comprising B-domain deleted factor VIII sequence variants. The rejection is respectfully traversed for the reasons described below, and it is submitted that the rejection should not be applied to claims 68-79 and 82-90

The Examiner argues that the specification is not enabling for the subject matter of claims 18, 19, and 35 because these claims "encompass a large amount of sequences which are unsequenced and untested, even [though] the assay methods are known in the art." September 11, 2002 Office Action, pages 6-7. The Examiner further states that "determination of the effects of [] particular sequence changes is not predictable until they are actually made and used, hence resulting in a trial and error situation." September 11, 2002 Office Action, page 7. Thus, the Examiner is requiring that the Applicants identify all variants of B-domain deleted factor VIII that meet the structural limitations of the claims and retain biological activity in order to satisfy the requirement for an enabling disclosure under 35 U.S.C. § 112, first paragraph.

The standard for enablement set forth in the Office Action is not supported by the applicable case law. Applicants note that an enabling disclosure must describe the claimed

invention in such a way as to enable the ordinarily skilled artisan to make and use the invention, and that this description be commensurate with the scope of the claimed invention. The test of enablement is not whether experimentation is necessary, but rather if experimentation *is* necessary, whether it is undue. *In re Angstadt*, 198 USPQ 214, 219 (C.C.P.A. 1976). The test of whether an invention requires undue experimentation is not based on a single factor, but rather is a conclusion reached by weighing many factors. *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). Factors to be considered in determining whether undue experimentation is required include the quantity of experimentation necessary, the amount of guidance provided in the specification, the presence of working examples of the invention in the application, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability in the art, and the breadth of the claimed invention. *Id.* at 1404. Accordingly, the holding of *Wands* does not require that an applicant identify every functional variant of the disclosed sequences so that no experimentation is required to make and use these variants as argued by the Examiner. Rather, the court in *Wands* set forth factors to be considered in determining whether undue experimentation is required to make and use the claimed invention.

As described on pages 8-9 of the Amendment mailed June 12, 2002, Applicants have provided guidance for determining the regions of B-domain deleted factor VIII that would tolerate modification. Based on the working examples of B-domain deleted factor VIII provided on lines 20-30 on page 20 of the specification and the guidance provided in the cited references regarding domains required for B-domain deleted factor VIII activity, a skilled artisan could choose among possible modifications to produce polypeptides within the structural parameters set forth in the claims and test these modified variants to determine if they retain B-domain deleted factor VIII biological activity. Making and testing such variants is routine to those of skill in the art. Furthermore, the scope of claims 18, 19, 35, and 68-79, and 82-90, is limited to variants that fall within defined structural parameters. These claims encompass only those nucleotide sequences having at least 75%, 80%, 85%, 90%, or 95% sequence identity with nucleotides 419-4835 of SEQ ID NO:1, and nucleotide sequences that hybridize to nucleotides 419-4835 of SEQ ID NO:1 under defined conditions, and the encompassed nucleotide sequences

are further limited by the functional limitation that they encode a biologically active B-domain deleted factor VIII.

Accordingly, when all of the factors set forth in *Wands* are considered together, it is clear that although some quantity of experimentation would be required to produce functional B-domain deleted factor VIII variants, the level of experimentation would not be undue in view of the nature of the invention, the state of the prior art (where factor VIII functional domains and activities have been described), the relative skill of those in the art (to whom the making and testing of variants is routine), the predictability in the art, the amount of direction provided in the specification (which provides guidance regarding preferred types of amino acid substitutions and describes assays for identifying functional B-domain deleted factor VIII polypeptides), the breadth of the claimed invention (for which the scope is defined by both structural and functional limitations), and the existence of a number of working examples of B-domain deleted factor VIII. These factors support the conclusion that one of skill in the art could practice the claimed invention without undue experimentation.

The Examiner cites Rudinger in *Peptide Hormones*, J.A. Parsons Ed. University Park Press, Baltimore, June 1976 in support of the rejection for lack of enablement, and quotes from page 6 of Rudinger, which states, "[t]he significance of particular amino acids and sequences for different aspects of biological activity cannot be predicted *a priori* but must be determined from case to case by painstaking experimental study." While the statements made by Rudinger regarding the correlation between amino acid sequence and polypeptide function may accurately reflect the views of those of skill in the art at the time of publication of this reference (*i.e.*, **1976**), this statement is not an accurate representation the view of those of skill in the art at the time the priority document for the present application was filed (*i.e.*, **1999**). The cited reference predates essentially the entire field of modern molecular biology. For example, the first recombinant bacterial plasmids were reported in the literature in 1973, only three years prior to the publication date of Rudinger. *See*, Cohen *et al.* (1973) *Proc. Natl. Acad. Sci. USA* 70:3240-4. Efficient methods for sequencing nucleotide sequences were not described until 1977, one year after the

publication date of the Rudinger reference. *See*, Maxam *et al.* (1977) *Proc. Natl. Acad. Sci. USA* 74:560-564). Accordingly, while making and testing protein variants may have required painstaking experimental study at the time that the Rudinger reference was published, this reference does not accurately reflect the state of the art for identifying functional protein variants in 1999 when the priority document for the present application was filed.

Applicants cite Gayle *et al.* (1993) *J. Biol. Chem.* 268:22105-22111, provided herewith as Appendix A for the convenience of the Examiner, to demonstrate the state of the art for making and testing variants of a polypeptide. Gayle *et al.* describe saturation mutagenesis of the mature human interluekin-1α (IL-1α) sequence. The authors report that more than 3,500 mutants of IL-1α were produced and analyzed to determine their biological activity and their binding activity, demonstrating that such mutants can be made and tested for function without undue experimentation. Furthermore, the authors state that "[m]ost of the molecule could be mutated with little effect on either [biological or binding] activity." Gayle *et al.*, page 22109, column 2. Thus, the Gayle *et al.* reference demonstrates that making functional variants of a polypeptide is routine to those of skill the art, and that many or most of the variants produced by amino acid substitution retain the biological activity of the native protein.

In view of the above arguments, all grounds for rejection under 35 U.S.C. §112, first paragraph have been overcome. Reconsideration and withdrawal of the rejections are respectfully requested.

The Rejection under 35 U.S.C. § 103 Should be Withdrawn

The Examiner indicates that the rejection of claims 1-64, 65, and 67 under 35 U.S.C. §103(a) as unpatentable over U.S. Patent No. 6,221,349 has been withdrawn in view of the Declaration under 37 CFR 1.131 submitted with the Office Action mailed June 12, 2002.

Claims 1, 3-18, 20, and 58-90 have been newly rejected under 35 U.S.C. § 103(a) on the grounds that they are unpatentable over U.S. Patent No. 6,221,646 in view of Robbins (1998)

Pharamcol. Ther. 80:35-47 and Pittman *et al.* (1993) *Blood* 81:2925-35 as evidenced by Vorachek *et al.* (2000) J.Biol. Chem. 275:29031-41. Claims 1-20 and 58-90 have also been rejected under 35 U.S.C. § 103(a) as unpatentable over U.S. Patent No. 6,332,459, Robbins *et al.*, and Pittman *et al.* in further view of U.S. Patent No. 6,258,595. The rejections are respectfully traversed.

Claims 1, 9, 18, 58, 68, 73, 80, and 82 have been amended to recite that the only promoter driving expression of the nucleotide sequence encoding B-domain deleted factor VIII is an AAV ITR. Support for these amendments may be found on lines 11-13 of page 18 of the specification. New claims 91 and 92 recite an rAAV vector comprising a heterologous nucleotide sequence encoding B-domain factor VIII operably linked with at least one enhancer and a promoter, wherein the promoter is an AAV ITR and the rAAV vector is capable of expressing B-domain deleted factor VIII at a level sufficient for treatment of a factor VIII associated-disorder. Support for these claims may be found in original claims 1 and 7, and in the specification on lines 17-18 of page 3, lines 1-3 of page 9, lines 3-9 of page 15, and lines 14-18 of page 18.

It is respectfully submitted that the rejection under 35 U.S.C. § 103 should not be applied to claims 1-20 and 58-90 as amended or to new claims 91 and 92 on the grounds that: 1) the prior art does not provide a motivating suggestion to produce an rAAV vector with a coding sequence for B-domain deleted factor VIII operably linked with an enhancer and an AAV ITR where the AAV ITR is the only promoter driving expression of the coding sequence for B-domain deleted factor VIII and the prior art does not reveal that in making such a vector, those of ordinary skill in the art would have a reasonable expectation that such a vector could be successfully used to produce B-domain deleted factor VIII at levels sufficient for treatment of a factor VIII-related disorder; and 2) the references cited in the Office Action do not establish a *prima facie* case of obviousness for the compositions of these claims.

The prior art does not teach or suggest the invention of claims 1-20 and 58-90.

The prior art teaches that expression of factor VIII from the human factor VIII cDNA is difficult because: 1) the hFVIII cDNA contains sequences that repress its expression; 2) FVIII is inefficiently transported from the endoplasmic reticulum to the Golgi system; and 3) FVIII is highly susceptible to proteolytic degradation in the blood. Therefore, any gene therapy strategy for the expression of FVIII must achieve high levels of FVIII expression in order to have a therapeutic effect. *See*, for example, the first full paragraph of the second column of page 155 of Chuah *et al.* (1998) *Critical Review in Oncology/Hematology* 28:153-71, provided as citation number 11 on Applicants' PTO form 1449 filed March 28, 2001.

Accordingly, prior to the Applicants' disclosure in the present application, those of skill in the art believed that in order to achieve sufficient expression of B-domain deleted factor VIII, a gene therapy vector for the expression of factor VIII must necessarily contain a strong promoter that could drive high levels of factor VIII transcription because factor VIII, and B-domain deleted factor VIII were known to be difficult to express. *See*, for example, Gnatenko *et al.* (1997) *Blood* 90:119a, Abstract 518-I, cited as reference 86 on Applicants' PTO Form 1449 filed February 21, 2003; and Ill *et al.* (1997) *Blood Coagulation and Fibrinolysis* 8:S23-S30, cited as reference 27 on Applicants' PTO Form 1449 mailed May 9, 2001. The requirement for a strong promoter when using an rAAV vector for expression of B-domain deleted factor VIII presents a technical obstacle because an insert containing a conventional strong promoter operably linked to the coding sequence for B-domain deleted factor VIII greatly exceeds the size limitations for efficient packaging by AAV as described by Robbins *et al.*, cited by the Examiner.

Furthermore, while it was recognized in the art that the AAV ITR has transcriptional activation activity (*see*, for example, U.S. Patent Number 5,587,308, cited as citation number 29 on Applicants' PTO Form 1449 mailed February 21, 2003) it was also recognized that this transcriptional activity is very low in comparison with the transcriptional activity of conventional promoters. *See,* for example, the top of column 1 of page 10159 of Zhang *et al.* (1998) *Proc. Natl. Acad. Sci. USA* 95:10158-63, provided herewith as Appendix B for the convenience of the

Examiner. As described above, the art taught that a strong promoter was required to drive expression of B-domain deleted factor VIII. *See*, Gnatenko *et al.*, and Ill *et al.* Accordingly, prior to the Applicants' disclosure, one of skill in the art would not have been led to construct a vector comprising an AAV ITR to drive expression of B-domain deleted factor VIII, because the art taught away from the use of a weak promoter such as the AAV ITR as the only promoter driving expression of B-domain deleted factor VIII.

Applicants are the first to disclose that an AAV ITR and an enhancer provide sufficient transcriptional activity to construct a useful vector for the expression of B-domain deleted factor VIII. None of the references, alone or in combination, teach or suggest an rAAV vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII operably linked with at least one enhancer and an AAV ITR, wherein the only promoter driving expression of the sequence encoding B-domain deleted factor VIII is the AAV ITR. It is the novel and surprising finding of the present invention that an AAV ITR combined with an enhancer is sufficient to drive the expression of B-domain deleted factor VIII in an animal model at a level recognized by those of skill in the art to be consistent with the treatment of factor VIII-associated disorders. In fact, the present application demonstrates that the claimed vectors are capable of expressing B-domain deleted factor VIII at levels sufficient to achieve a therapeutic effect in an animal model. *See*, for example, Figures 4 and 5, and Examples 11 and 12 on pages 47-48 of the specification. Accordingly, claims 1-20 and 58-90 are patentable under 35 U.S.C. § 103 because they recite a combination of elements that was neither taught nor suggested by the art.

New claims 91 and 92 recite a recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII operably linked with at least one enhancer and an AAV ITR, wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV and said rAAV vector is capable of expressing B-domain deleted factor VIII at a level sufficient for treatment of a factor VIII associated-disorder. As described above, the Applicants' disclosure is the first to demonstrate that an expression construct comprising an AAV ITR and an enhancer, where the AAV ITR is

the only promoter driving expression may be used to express B-domain deleted factor VIII at levels sufficient for treatment using a factor VIII-related disorder.

The references cited in the Office Action do not establish a prima facie case of obviousness for claims 1-20 and 58-92.

U.S. Patent No. 6,221,646 teaches an rAAV vector that comprises an albumin promoter in some embodiments. The patent describes the production of rAAV stocks having up to 7.4×10^8 infectious particles. These stocks are made from rAAV vectors containing an ampicillin resistance gene. The patent suggests that the vector could be used to express full-length factor VIII. The patent also teaches that large vectors cannot be packaged by AAV. This patent describes an embodiment in which the rAAV vectors are used to express full-length factor VIII, but does not suggest the use of rAAV vectors to express B-domain deleted factor VIII and does not teach or suggest that B-domain deleted factor VIII can be successfully expressed at levels sufficient for treatment using the disclosed vectors.

Robbins *et al.* teach that current methods of AAV preparation can result in stocks of up to 10^{14} particles per ml, although the actual number of infectious virus particles in such preparations is unclear. Robbins *et al.* also teach that one disadvantage of using AAV vectors for gene therapy is that vectors larger than 5.2 kb are not efficiently packaged, and thus AAV can only be used for transfer of inserts smaller than 5 kb. *See*, Robbins *et al.*, page 40, column 2. Robbins *et al.* do not teach that an expression cassette containing B-domain factor VIII could be expressed at levels sufficient for treatment using AAV vectors. In fact, the Robbins *et al.* reference teaches that expression cassettes such as those claimed in the present invention are too large to be efficiently packaged and expressed by AAV.

Pittman *et al.* teach the sequence and properties of a B-domain deleted factor VIII. The authors state that their goal for producing B-domain deleted factor VIII was to generate a smaller molecule that could be more efficiently expressed and that would have reduced heterogeneity. While the authors suggest that B-domain deleted factor VIII may be useful in gene therapy, they

do not teach that the coding sequence for B-domain deleted factor VIII could be successfully expressed at levels sufficient for treatment using an rAAV vector.

U.S. Patent No. 6,258,595 describes an rAAV vector comprising a spacer sequence interposed between the promoter and the rep gene ATG start site. This patent does not suggest that B-domain deleted factor VIII can be expressed at levels sufficient for treatment using an rAAV vector.

In *In re Vaeck,* 20 USPQ2d 1438 (Fed. Cir. 1991), the Federal Circuit held that:

> Where claimed subject matter has been rejected as obvious in view of a combination of prior art references, a proper analysis requires, *inter alia*, consideration of two factors: (1) whether the prior art would have suggested to those of ordinary skill in the art that they should make the claimed compositions or device, or carry out the claimed process; and (2) whether the prior art would also have revealed that in so making and carrying out, those of ordinary skill would have a reasonable expectation of success. Both the suggestion and the reasonable expectation of success must be found in the prior art, not in the applicant's disclosure.

In re Vaeck at 1442, *citing In re Dow Chemical Co.*, 5 USPQ2d 1459, 1531 (Fed. Cir. 1988). Furthermore, the mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *Manual of Patent Examining Procedure* § 2143.01 (8th ed.), citing *In re Mills*, 16 USPQ2d 1430 (Fed. Cir. 1990).

In the present case, no *prima facie* case of obviousness has been established because the art cited in the Office Action does not provide the suggestion to those of ordinary skill in the art to make the claimed compositions. The cited references do not suggest the desirability of using the rAAV vector described in U.S. Patent No. 6,221,646 to express the B-domain factor VIII of Pittman *et al.* U.S. Patent No. 6,221,646 describes an embodiment in which the rAAV vectors are used to express full-length factor VIII, but does not suggest the use of rAAV vectors to express B-domain deleted factor VIII. Furthermore, one of skill in the art would have no reasonable expectation that the B-domain deleted factor VIII of Pittman *et al.* could be expressed at levels sufficient for treatment of a factor VII-associated disorder using the rAAV vectors

described in U.S. Patent No. 6,221,646, because, prior to the Applicants' disclosure, the art taught that B-domain deleted factor VIII was too large to be expressed using rAAV vectors. *See*, for example, Russell and Kay (1999) *Blood* 864-874, cited on Applicants' PTO Form 1449 mailed March 28, 2001, which teaches that "B-domain deleted factor VIII message is about 4.4 kb, making it very difficult to produce a high-titer AAV vector with a promoter and a polyadenylation site." Russell and Kay, page 868, column 1, second paragraph. Accordingly, the references cited in the Office Action do not provide a motivating suggestion to produce an rAAV vector with a coding sequence for B-domain deleted factor VIII.

Accordingly, the prior art provides no motivation to produce rAAV vectors comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII operably linked to an enhancer and an AAV ITR, where the only promoter is an AAV ITR. One of skill in the art would have no reasonable expectation that such a vector could be used successfully in gene therapy because the prior art taught that the AAV ITR has weak transcriptional activity and that the therapeutic expression of factor VIII requires the use of a promoter having strong transcriptional activity in order to obtain therapeutic levels of FVIII in the blood stream. Thus, it is the Applicants' disclosure, which shows that an AAV ITR has sufficient transcriptional activity to produce levels of factor VIII sufficient for treatment of a factor VIII-related disorder, rather than the prior art, which provides the motivation to produce the compositions of claims 1-20, 58-77, 79-88, and 90-92.

In view of the above arguments, all grounds for rejection under 35 U.S.C. § 103(a) have been overcome. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

CONCLUSIONS

It is believed that all the rejections have been obviated or overcome and the claims are in conditions for allowance. Early notice to this effect is solicited.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 16-0605.

Respectfully submitted,

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Nora C. Martinez

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Version with Markings to Show Changes Made:

In the claims:

Please amend claims 1, 9, 12, 18, 58-65, 68, 73-77, 79-80, and 82 as follows:

1. (Amended) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII operably linked with at least one enhancer and [at least one promoter]an AAV ITR, wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR.

9. (Amended) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding B-domain deleted factor VIII operably linked with a liver-preferred enhancer[expression control element] and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR.

12. (Amended) The rAAV vector of claim 9, wherein said liver-preferred expression control element comprises the hepatitis B virus EnhI enhancer given as about nucleotides 150-278[419 to 4835] of the nucleotide sequence set forth in SEQ ID NO:1.

18. (Twice amended) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence encoding a B-domain deleted factor VIII operably linked with an enhancer[,] and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR and wherein said heterologous nucleotide sequence is selected from the group consisting of:

(a) the nucleotide sequence given as nucleotides 419 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1,

(b) a nucleotide sequence that hybridizes to the nucleotide sequence of (a) under conditions of high stringency and which encodes a biologically active B-domain deleted factor VIII, wherein the conditions of high stringency comprise hybridization in 25% formamide and 5X SSC at 42°C and at least one wash in 0.3 M NaCl, 0.03 M sodium citrate at 60°C; and

(c) a nucleotide sequence that that differs from the nucleotide sequences of (a) and (b) above due to the degeneracy of the genetic code, and which encodes a biologically active B-domain deleted factor VIII.

58. (Amended) A nucleic acid molecule comprising a nucleotide sequence encoding B-domain deleted factor VIII operably linked with a hepatitis virus enhancer[expression control element] and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR.

59. (Amended) The nucleic acid molecule[nucleotide sequence]of claim 58, wherein said hepatitis virus enhancer[expression control element] is from a hepatitis B virus.

60. (Amended) The nucleic acid molecule[nucleotide sequence] of claim 59, wherein said hepatitis virus enhancer[expression control element] is a hepatitis B virus EnhI or EnhII enhancer.

61. (Amended) The nucleic acid molecule[nucleotide sequence] of claim 60, wherein said hepatitis virus enhancer[expression control element] is a hepatitis B virus EnhI enhancer.

62. (Amended) The nucleic acid molecule[nucleotide sequence] of claim 58, wherein said nucleic acid molecule[nucleotide sequence]comprises the sequence given as about nucleotides 150 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.

63. (Amended) The nucleic acid molecule[nucleotide sequence] of claim 62, wherein said nucleic acid molecule[nucleotide sequence] further comprises [a promoter and]a polyadenylation sequence.

64. (Amended) The nucleic acid molecule[nucleotide sequence] of claim 63, wherein said nucleic acid molecule[nucleotide sequence] comprises the sequence given as nucleotides 150 to 4914 of the nucleotide sequence set forth in SEQ ID NO:1.

65. (Amended) A vector comprising the nucleic acid molecule[nucleotide sequence]of claim 58.

68. (Amended) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence operably linked with an enhancer and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR and wherein said heterologous nucleotide sequence is at least 75% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

73. (Amended) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence operably linked with a hepatitis virus enhancer and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR and[expression control element,]wherein said heterologous nucleotide sequence is at least 75% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.

74. (Amended) The rAAV vector[nucleotide sequence] of claim 73, wherein said hepatitis virus enhancer[expression control element] is from hepatitis B virus.

75. (Amended) The rAAV vector[nucleotide sequence]of claim 74, wherein said hepatitis virus enhancer[expression control element] is a hepatitis B virus EnhI or EnhII enhancer.

76. (Amended) The rAAV vector[nucleotide sequence] of claim 75, wherein said rAAV vector[nucleotide sequence]comprises the sequence given as about nucleotides 150 to 4835 of the nucleotide sequence set forth in SEQ ID NO:1.

77. (Amended) The rAAV vector[nucleotide sequence] of claim 73, wherein said rAAV vector [nucleotide sequence] further comprises [a promoter and]a polyadenylation sequence.

79. (Amended) A cell comprising the rAAV vector of claim 77[78].

80. (Amended) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence operably linked with an enhancer and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR and[,] wherein said heterologous nucleotide sequence encodes the amino acid sequence set forth in SEQ ID NO:2.

82. (Amended) A recombinant adeno-associated virus (rAAV) vector comprising a heterologous nucleotide sequence operably linked with at least[lease] one enhancer and an AAV ITR wherein the only promoter driving expression of said B-domain deleted factor VIII is the AAV ITR and [at least one promoter,]wherein said heterologous nucleotide sequence is at least 75% identical to nucleotides 419-4835 of SEQ ID NO:1 and encodes a biologically active B-domain deleted factor VIII.